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DEVELOPMENTAL BIOLOGY/MORPHOGENESIS

Use of morphometric parameters for tracking ovule and microspore evolution in grapevine (*Vitis vinifera* L., cv. "Aragonez") and evaluation of their potential to improve *in vitro* somatic embryogenesis efficiency from gametophyte tissues

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Abstract Somatic embryogenesis induction from *in vitro* cultured stamens and carpels is highly dependent on explants' inoculation at specific developmental stages. To establish good correlations between measurable morphometric parameters of flowers or flower buds and developmental stages of micro- and macrosporogenesis, this procedure is the easiest way to simplify the *in vitro* culture procedures. These correlations were established here for the most important Iberian grapevine cultivar, the "Aragonez", named "Tempranillo" in Spain and "Tinta Roriz" in the north of Portugal, and were based in floral buds and anther measurements. The anther length, with a correlation coefficient of 0.90, proved to be the best morphometric parameter to follow microsporogenesis evolution. A correlation between micro- and macrosporogenesis evolutionary stages was also positively established, allowing the use of morphometric parameters for tracking ovule evolution as well. Carpels in several evolutionary stages were *in vitro* cultured to evaluate the aging effect on the capacity for somatic embryogenesis induction. Explants inoculated in the earliest stages of macrosporogenesis presented the best results. Media culture formulations were also tested for ovary culture, with the best results being achieved with a 5:1 auxin/cytokinin ratio.

Keywords *Vitis vinifera* · Grapevine · Microsporogenesis · Macrosporogenesis · Somatic embryogenesis

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Introduction

The first reports dealing with *in vitro* somatic embryogenesis in grapevine arise from the late 1970s (Hirabayashi et al. 1976; Rajasekaran and Mullins 1979). Since then, successful protocols for somatic embryo induction and its conversion into plants were developed for several *Vitis vinifera* L. cultivars (Rajasekaran and Mullins 1983; Mauro et al. 1986; Stamp and Meredith 1988; Mozsár and Viczián 1996; Nakano et al. 1997; Jayasankar et al. 1999; Cardoso et al. 2001; Martinelli et al. 2001; Leal et al. 2004; Cardoso 2006; Leal et al. 2006; Gambino et al. 2007; Pinto-Sintra 2007). Among all the factors able to interfere with the success of these protocols, the physiological stage of the primary explants is probably the most important one.

The tetrad and the uninucleate stages of microspore evolution are usually reported as the most responsive ones for anther culture (Rajasekaran and Mullins 1979; Hirabayashi and Akihama 1982; Mauro et al. 1986; Salunkhe et al. 1999;